THE INFLUENCE OF LIGHT AND TEMPERATURE ON BODY FAT AND REPRODUCTIVE CONDITIONS OF *RANA PIPIENS*[1, 2]

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ABSTRACT

Specimens of *Rana pipiens* were subjected to different environmental conditions in order to determine the role of light and temperature on the amount and pattern of fat utilization, as well as the effects of these factors on the reproductive condition of the species. Temperature influences the amount of fat utilization; however, both light and temperature influence the pattern of fat utilization and the reproductive condition. A correlation was found between the amount and pattern of fat utilization or deposition and the reproductive condition of individuals.

INTRODUCTION

An inverse relationship has been shown to exist between the amount of body fat and the gonadal weight in many species of vertebrates. In this regard, Hahn and Tinkle (1965) demonstrated that the fat cycle in *Uta stansburiana* was associated with the reproductive cycle of the species. Volsøe (1949) stated that fat bodies contributed to the rapid growth of the eggs during pregnancy in *Vipera berus*, and Tinkle (1962) concluded that female *Crotalus atrox* have biennial egg cycles and that emergence from hibernation may not result in reproduction unless considerable fat storage has occurred. Tinkle also found that females due to mate in the spring have 50 percent more fat than those which have given birth the previous fall. Brenner (1966) stated that the amount of body fat and the percent of the total fat present in the fat bodies of *Rana clamitans* and of *Acris crepitans* was inversely correlated with gonadal weight. The objective of the current study was to investigate the role of light and temperature on the amount and pattern of fat utilization, as well as the relationship between body fat and the reproductive cycle of *Rana pipiens*. The term hibernation in this paper is defined as "overwintering" in a state of reduced metabolic activity.

METHODS

Frogs (*Rana pipiens*) were obtained from a commercial dealer in Pittsburgh, Pennsylvania, in mid-September and maintained under conditions of natural daylight and temperature for one month prior to the initiation of the experiment. During this period, natural food was available to the populations. Frogs were housed in groups of five in plastic containers 12 x 6 x 3 inches in size, filled with ½ inch of mud and 2 inches of water. Water was added whenever necessary to maintain the same volume of water in all containers. Experimental populations were maintained without food at four different environmental conditions: (1) L/RT: natural daylight at an average temperature of 22°C (SE 0.21 C); (2) D/RT: complete darkness at an average temperature of 22°C (SE 0.25 C); (3) L/C: eight hours of light at an average temperature of 5°C (SE 0.13 C); (4) D/C: complete darkness at an average temperature of 5°C (SE 0.21 C).

The total body fat, percent of the total fat in the fat bodies, and the weight of the gonads were determined at the beginning of the experiment, and at regular intervals throughout the experimental period. Individuals were removed from the experimental population at random and the testis or two samples of eggs were

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removed from the animal. The testis and one sample of eggs were placed in 10% formalin for weighing and histological examination, while the other egg sample was frozen at −20°C and saved for fat determination. The fat bodies were removed, dehydrated, and placed back in the animal. Individuals were dried at 70°C until no further weight loss occurred, usually 24 hours, and then placed in petroleum ether for 24 hours. The difference between the dry weight before and after extraction was the amount of body fat (Brenner and Malin, 1965).

The testes and eggs were weighed to within ±0.1 mg. Ten eggs were removed from formalin, dried on a paper towel, weighed individually and the mean used as the average egg weight for the particular specimen. The testes were sectioned at 5 μ and stained with hematoxylin and eosin for spermogenesis, and with sudan IV for Leydig cell activity. The amount of spermogenic and sudanophilic activity was classified by a scale ranging from 0 to 5 (0=no activity; 1=1 to 20%; 2=21 to 40%; 3=41 to 60%; 4=61–80%; 5=81 to 100%). All weighing was within an accuracy of ±0.1 mg.

During the second phase of the study, the influence of testosterone on the amount and pattern of fat deposition was investigated. Frogs obtained in mid-February were placed in complete darkness at 5°C for 14 days and then removed to be either castrated or sham-operated by opening the body cavity. The animals were then returned to the hibernation chamber for seven days. Therefore, hibernation conditions were maintained for a total of 21 days prior to the first injection of testosterone. Frogs were then removed from the hibernation chamber and divided into three groups as follows: sham-operated, injected with 0.05 cc of physiological saline (n=12); castrate, injected with 0.05 cc physiological saline (n=8); and castrate, injected with 1 mg of an aqueous suspension of testosterone (n=11). All injections were given daily for five days and the animals were fed *ad lib.* At the termination of the experiment, the total body fat and percent of the total body fat in the fat bodies, as well as the reproductive condition of the sham-operated controls, was determined by the procedures outlined previously. The amount and pattern of fat deposition and the reproductive condition were also determined for ten individuals exposed simply to 21 days of hibernating conditions at 5°C. In order to simulate natural conditions of the breeding season during the period of testosterone injections, all three populations were maintained at an average environmental temperature of 17°C with a 14-hour photoperiod. These temperature and light conditions are similar to those of the breeding season in the Greenville, Pennsylvania, area (Brenner, 1966).

RESULTS

Fat Utilization

Temperature influences the rate of fat utilization, but both light and temperature may alter the pattern of fat utilization. Frogs maintained under light and dark conditions at room temperature (23°C) utilized their body fat at a significantly faster rate than did frogs exposed to similar conditions at 5°C ($P < .001$) (fig. 1). Light did not significantly influence the rate of fat utilization at room temperature or at 5°C ($P > .40$). There was no significant difference between the rates of fat utilization of male and female frogs under any environmental condition ($P > .40$).

The percent of total fat in the fat bodies decreased at a significantly faster rate in both males and females exposed to complete darkness at room temperature than in those exposed to complete darkness at 5°C ($P < .01$). However, the percent of the total fat in the fat bodies decreased at a significantly faster rate in females maintained in natural light at room temperature than in those kept in eight hours of light at 5°C ($P < .05$); a similar reaction did not occur in males ($P > .50$) (fig. 2). The percent of total body fat present in the fat bodies decreased

at a significantly faster rate in frogs maintained in complete darkness at room temperature than in those maintained in a similar condition at 5°C ($P < .001$). Light appeared to affect the pattern of fat utilization in frogs at 5°C. Males exposed to 8 hours of light at 5°C utilized their fat bodies at a significantly faster

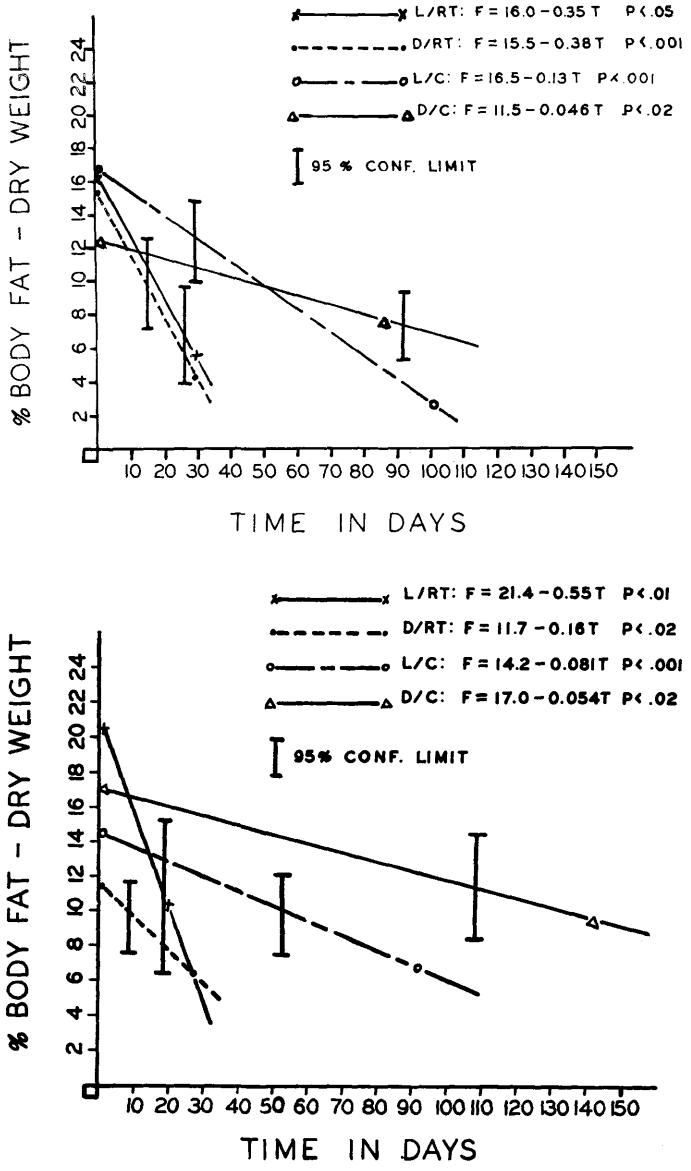

FIGURE 1. The decrease in the total body fat (F) during the time (T) that *Rana pipiens* were exposed to the different environmental conditions (Top: male, n=77; Bottom: female, n=76). L/RT: natural daylight at an average temperature of 22°C (SE 0.21); D/RT:complete darkness at an average temperature of 22°C (SE 0.25); L/C: eight hours of light at an average temperature of 5°C (SE 0.13); D/C: complete darkness at an average temperature of 5°C (SE 0.21).

rate than those maintained in complete darkness at 5°C ($P<.01$), but a similar phenomenon did not occur in females ($P>.10$). Light did not appear to influence the percent of the total fat in the fat bodies in frogs maintained at room temperature.

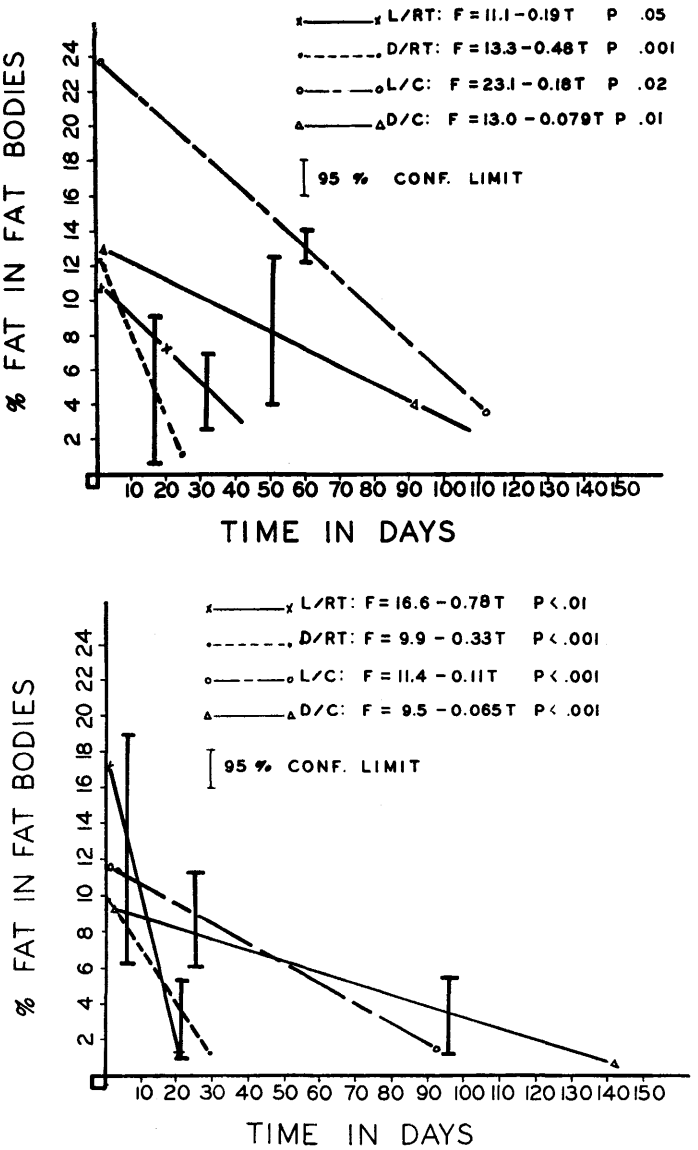

FIGURE 2. The decrease in the percent of the total body fat in the fat bodies (f) during the time (T) that *Rana pipiens* were exposed to the different environmental conditions (Top: male, n=77; Bottom: female, n=76). L/RT: natural daylight at an average temperature of 22°C (SE 0.21); D/RT: complete darkness at an average temperature of 22°C (SE 0.25); L/C: eight hours of light at an average temperature of 5°C (SE 0.13); D/C: complete darkness at an average temperature of 5°C (SE 0.21).

Light influenced the pattern of fat deposition in males, since both those exposed to complete darkness at room temperature and those kept at 5°C had less fat in their fat bodies than did those at the initiation of the experiment ($P<.01$) (Table 1). However, a similar reaction did not occur in those individuals exposed to light at room temperature or at 5°C ($P>.30$). The percent of total body fat in the fat bodies decreased significantly in females exposed to all four environmental conditions from that at the initiation of the experiment ($P<.01$).

TABLE 1

Percent of the total fat in the fat bodies and the weight of the gonads of Rana pipiens *subjected to different environmental conditions as sampled at random intervals*

Condition	Males					Females				
	Number	% of Total Fat in Fat Bodies		Testes Wt. mg.		Number	% of Total Fat in Fat Bodies		Eggs Wt. mg.	
		Mean	SE	Mean	SE		Mean	SE	Mean	SE
Start	10	9.9	1.02	39.4	10.80	10	9.6	1.02	0.81	0.50
L/RT	17	7.0	2.46	38.5	8.05	14	4.9	1.39	1.20	0.61
D/RT	16	4.3	1.42	51.1	4.74	10	4.2	2.72	1.80	0.34
L/C	20	10.9	7.08	50.8	4.11	19	1.6	1.26	1.50	0.38
D/C	14	2.5	0.55	44.1	3.07	23	1.9	0.88	2.00	0.60

Reproductive Condition

Light and temperature may also affect the development of the gonads in male frogs (Bebak, 1958; Tchou and Wang, 1963). The weight of the testes of frogs exposed to conditions of complete darkness at room temperature or to eight hours of light at 5°C were significantly heavier than were the testes of individuals sampled at the initiation of the experiment ($P<.01$); however, the testis weight of individuals at the initiation of the experiment did not vary significantly from those exposed to conditions of natural daylight at room temperature or complete darkness at 5°C ($P>.40$). Likewise, the weights of testes of frogs exposed to natural daylight at room temperature were significantly less than those of frogs exposed to a similar temperature condition and complete darkness ($P<.01$) and those exposed to eight hours of light at 5°C ($P<.01$). The testis weight of frogs exposed to complete darkness at 5°C was significantly less than that of those exposed to similar conditions at room temperature ($P<.01$). The weight of the testis did not vary significantly between individuals exposed to the other environmental conditions ($P>.30$). The weight of the individual eggs did not vary significantly between individuals at the initiation or among the different environmental conditions ($P>.40$).

The amount of spermatogenic activity also appeared to be influenced by both light and temperature. Frogs exposed to eight hours of light at 5°C had fewer mature spermatozoa present in their testes than did individuals exposed to the other environmental conditions. A reduction in the number of individuals containing mature spermatozoa occurred in individuals exposed to 5°C under both light and dark conditions; however, a greater reduction occurred in individuals exposed to eight hours of light at 5°C than in those exposed to complete darkness. Light and temperature also affected the amount of sudanophilic activity. Such activity decreased under both light and dark conditions at 5°C, as well as under conditions at room temperature (Table 2).

A relationship also existed between the size of the gonads and the amount of

body fat, as well as with the percent of the total body fat in the fat bodies. The total body fat and the amount of fat in the fat bodies decreased as the weight of the testes increased (percent of body fat, $P<.01$; percent of total fat in fat bodies, $P<.001$). A significant correlation also occurred between egg size and the amount of body fat, and the percent of total fat in the fat bodies (percent body fat, $P<.01$; percent total fat in fat bodies, $P>.001$). The fat content of the eggs (F) increased at the rate of 4.18 as the weight (W) of the eggs increased ($P<.001$). These data support the fact that the amount and pattern of fat utilization in frogs may be related to the reproductive cycle of the species.

TABLE 2

Reproductive condition of Rana pipiens *subjected to different environmental conditions as sampled at random intervals*

Condition	Number	Spermatogenesis		Sudanophilic Activity		% of Individuals Containing Spermatozoa
		Mean	SE	Mean	SE	
Start	6	3.5	0.91	3.8	1.00	100.0
L/RT	17	3.6	0.32	3.6	0.19	100.0
D/RT	16	3.6	0.30	2.9	0.31	100.0
L/C	20	1.1	0.46	2.4	0.37	63.2
D/C	14	3.4	0.49	1.8	0.10	88.9

Castration Experiment

The results of this phase of the study indicated that the reproductive condition of frogs appears to have a greater influence on the pattern of fat deposition than it does on the amount of body fat present in the individual. All the experimental individuals had significantly more body fat than did individuals sampled at the initiation of the experiment, indicating that the frogs were depositing fat throughout the five-day experimental period ($P<.03$), although there was no significant difference in the amount of body fat among the three experimental groups ($P>.50$) (Table 3). However, the reproductive condition of the individuals appeared to influence the pattern of fat deposition, for both sham-operated and castrated individuals injected with testosterone had significantly less fat in their fat bodies than did either castrated-saline-injected individuals or frogs sampled at the initiation of the experiment ($P<.001$). The individuals that were castrated and injected with saline also had significantly less fat in their fat

TABLE 3

The amount and pattern of fat deposition in male Rana pipiens *subjected to different experimental conditions*

Condition	Number	Body Fat (mg)		% Body Fat Dry Weight (mg)		% of Total Body Fat in the Fat Bodies		Testes Weight	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Start	10	323.5	52.2	3.8	0.37	33.5	4.7	42.4	1.1
Sham-operated	12	496.3	71.4	6.3	0.87	10.2	1.1	39.3	2.2
Castrated-testosterone	11	514.6	54.4	6.5	0.52	10.5	2.3	—	—
Castrated-saline	8	419.1	68.0	6.3	1.00	25.1	4.9	—	—

bodies than did those sampled at the initiation of the experiment ($P < .02$). There was not, however, a significant difference in the percent of total body fat in the fat bodies between the sham-operated and the castrated individuals injected with testosterone ($P > .50$). Individuals that were castrated and injected with saline had significantly less fat in their fat bodies than did individuals castrated and injected with testosterone ($P < .001$); hence testosterone appears to affect the pattern of fat deposition, but not the total amount of body fat.

Further evidence that the reproductive cycle of the individual influences the pattern of fat deposition was shown by a histological examination of the testes. There was no significant difference between the weight of the testes or the amount of mature spermatozoa present in the testes of the sham-operated frogs and those sampled at the initiation of the experiment; however, there was an increase in testosterone production, as indicated by sudanophilic activity, in the sham-operated individuals, as compared with those sampled at the initiation of the experiment. This phenomenon was probably caused by the increased temperature and photoperiod during the experimental period (17°C and 14 hours of light), which in turn influenced the pattern of fat deposition.

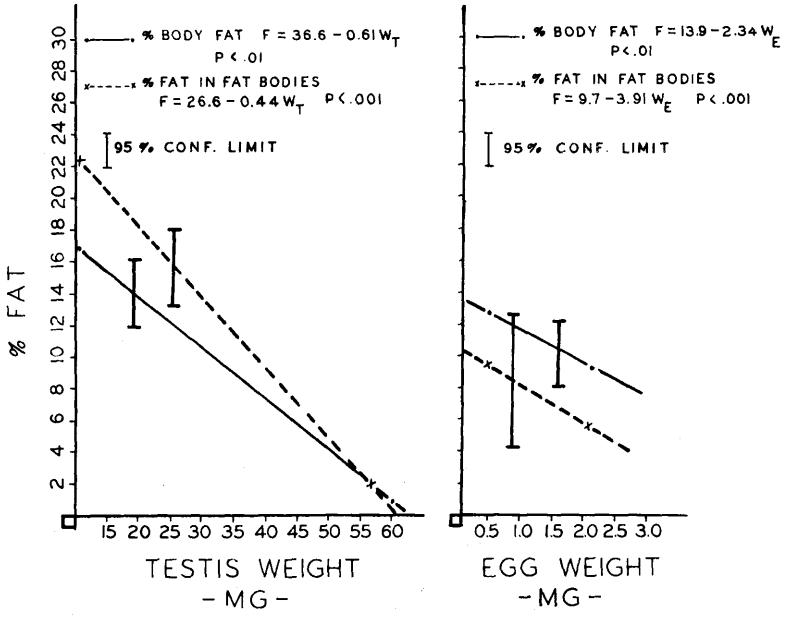

FIGURE 3. The relationship between the weight of the gonads (Testis, W_T; Eggs, W_E) and total body fat (F) and percent of the total body fat in the fat bodies (F) of *Rana pipiens*.

DISCUSSION

During the past two decades, several investigators have shown the existence of a correlation between the fat cycle and the reproductive cycle of the species. Smith (1950) reported that rapid depletion of fat and lipid stores occurred in *Rana temporaria* during the breeding season, while Mizell (1965) stated that the fat-body weight of *Rana pipiens* was at its minimum in June, increasing rapidly in July to reach its peak in August. Mizell also reported that the fat bodies decreased rapidly during September and October, followed by a gradual decrease

in weight in November that continued throughout the winter and spring. In this regard, Bush (1963) stated that the fat bodies in *Bufo fowleri* were the smallest during the breeding season, increasing in size during June, and reaching their maximum size prior to hibernation. Brenner (1966) found that male green frogs, *Rana clamitans*, and cricket frogs, *Acris crepitans*, possessed mature sperm one week after emerging from hibernation, with sudanophilic activity of the testis increasing during the breeding season so that it reached its peak during the period of rapid utilization of the body fat. A similar phenomenon appears to occur in several species of reptiles (Volsøe, 1949; Tinkle, 1962; Hahn and Tinkle, 1965; Berry and Lim, 1967).

The results of this study indicate that both light and temperature influence both the rate and pattern of fat utilization and the reproductive cycle of the species during hibernation. Frogs maintained at room temperature under both light and drak conditions utilized their body fat at a significantly faster rate than those maintained under similar conditions at 5°C. However, a variation did occur in the pattern of fat utilization in frogs maintained under different environmental conditions. The interaction between light and temperature may also affect the reproductive condition of males, as indicated by a reduction of both spermatogenic and sudanophilic activity in *Rana pipiens* subjected to eight hours of light at 5°C. The data obtained during this study also suggest that a relationship exists between the body fat and the reproductive condition of the animal. The amount and pattern of fat utilization was correlated with the size of the gonads; however, at the present time it is not known whether this phenomenon is due to a direct effect of the gonads on fat utilization or an indirect effect caused by other physiological changes within the animal. The data obtained on the sham-operated and castrated individuals injected with testosterone indicated that testosterone affected the pattern of fat deposition, but not the total amount of body fat present in the animal.

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